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Short communication

Effects of fluconazole on the pharmacokinetics and pharmacodynamics of antimony in cutaneous leishmaniasis-infected hamsters

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Abstract

Pentavalent antimony (Sb^V) compounds are the drugs of choice for the treatment of all forms of leishmaniasis. For 20 years there has been an interest in antifungal azoles for treating leishmaniasis, with variable success. In the current study, we examined the effects of co-administration of fluconazole (FLZ) on the pharmacokinetics and pharmacodynamics of Sb^V in cutaneous leishmaniasis-infected hamsters. Hamsters were divided into four groups. All hamsters were injected with 0.1 mL of 1×10^8 promastigotes/mL into the right foot on Day 1. Treatment was started 5 days after the infection. The antimony group received 80 mg/kg/day of Pentostam® intramuscularly whilst the FLZ group received FLZ 20 mg/kg/day orally for 14 days. The combination group received both Pentostam® and FLZ at the above mentioned doses for 14 days. Animals in the control group received no treatment. The infected footpads were measured on Days 1 and 14. A pharmacokinetic study was conducted on Days 1 and 14 of treatment, representing single- and multiple-dose pharmacokinetics, respectively. Blood samples were collected at different time intervals up to 24 h. Sb^V was determined using flameless atomic absorption spectrophotometry. Pharmacokinetic parameters were calculated using a non-compartmental analysis. In the single-dose study, there was no statistically significant difference in any of the pharmacokinetic parameters of Sb^V when given alone or with FLZ. However, on Day 14 a significant increase in peak plasma concentration (C_{max}) (three-fold) and area under the concentration–time curve (AUC) (four-fold) of antimony was observed when Sb^V was co-administered with FLZ. A statistically significant prolongation of the terminal half-life from 1.63 to 8.67 h ($P < 0.05$) was also observed. A significant reduction in clearance was detected. However, FLZ had no effect on the pharmacodynamics of Sb^V as measured by footpad sizes. In conclusion, FLZ did not improve the therapeutic effect of Sb^V when given concomitantly despite the significant increase in blood concentration and prolongation of the elimination half-life of Sb^V.

Keywords: Antimony; Sodium stibogluconate; Leishmaniasis; Pharmacokinetics; Fluconazole

1. Introduction

Leishmaniasis is a devastating protozoan disease caused by flagellated organisms of the genus *Leishmania*, which infect man by transmission via the bite of a sandfly. Cutaneous leishmaniasis (CL) is caused by the species *Leishmania major*. Most CL lesions manifest as indolent ulcers on exposed skin. Over 90% of cases heal spontaneously within 3–18 months depending upon the infecting species and the host's immune response.

For six decades, long parenteral courses of pentavalent antimonial drugs have proved to be effective antileishmanial agents and have been used for all forms of leishmaniasis, but their utility is limited by their cost, toxicity and inconvenience [1]. Sodium stibogluconate (Pentostam®) is a complexed pentavalent antimony (Sb^V) mainly used in patients with disfiguring or relapsing cutaneous or mucocutaneous leishmaniasis [2]. Second-line drugs include amphotericin B and pentamidine, both of which are nephrotoxic. The difficulties of treatment are exacerbated by the spread of resistance to

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antimony in India and the intractability of the disease to all drugs in patients co-infected with human immunodeficiency virus (HIV) [3].

Over the last few years there have been a number of reports of new therapies, but none has proved to be effective, safe and easily administered [4]. Imiquimod, an immunomodulator for genital warts, produced a 90% cure rate when the ointment was used in conjunction with antimonials in 12 patients who had not responded to antimony alone [5].

For 20 years there has been an interest in antifungal azoles for treating leishmaniasis. Owing to the similarity between the *Leishmania* and fungal cell membranes, these agents have potential in the treatment of leishmaniasis. Ketoconazole and itraconazole have been used to treat CL with variable success [6,7].

The mechanism of action of the azoles is inhibition of cytochrome P450-dependent ergosterol synthesis, which results in the inhibition of conversion of lanosterol to ergosterol and subsequently to inhibition of cell membrane formation.

Most trials have been limited and results are equivocal. Recently, the use of 200 mg/kg fluconazole (FLZ) for 6 weeks led to healing of CL (*L. major*) in 79% of patients compared with 34% receiving placebo [8].

It is theorised that combining FLZ with Sb^V would produce a synergistic therapeutic effect that would heal CL more completely and in a shorter period, thus reducing exposure of patients to the toxicities and difficulties of using Sb^V.

There are no clinical data available on the possible interaction of Sb^V and FLZ. It is well known that different diseases affect the pharmacokinetics of drugs in general. We have some evidence indicating that *Leishmania* affects the clearance of antimony in hamsters (unpublished data). Therefore, this study was undertaken to investigate the effect of administration of single and multiple doses of FLZ on the pharmacokinetics of Sb^V in CL-infected hamsters. In addition, the therapeutic effect of FLZ on healing of a CL ulcer in the footpad of hamsters when administered alone and in combination with Pentostam® was studied.

2. Materials and methods

2.1. Parasite

L. major parasites were preserved in liquid nitrogen with subsequent culturing until they were used for infecting hamsters. At that time, promastigotes were grown in 3N biphasic medium until they reached their stationary phase, i.e. ≥4 days.

2.2. Animals

Age-matched Syrian hamsters with an average weight of 140 ± 5 g (institution animal house) were used. There were 18 hamsters in each treatment group and 6 in the control group. Animals were housed six per cage with free access to food and water. Animal handling complied fully with our institutional policy.

Each hamster was injected with 0.1 mL of 1×10^8 promastigotes/mL into the right foot. This infective dose was selected to ensure development of a significant lesion. The day of infection was considered Day 1. Treatment was started on Day 5 post-infection and continued for 14 days.

2.3. Drugs

Pentostam® (100 mg/mL antimony; Burroughs Wellcome, Research Triangle Park, NC) was administered intramuscularly. FLZ (Pfizer, Memphis, TN) was purchased as Diflucan® and was administered orally as a suspension. All other chemicals and solvents used in the analysis of antimony were of analytical grade.

2.4. Single- and multiple-dose pharmacokinetic protocol

Three groups of 18 hamsters were selected randomly to receive either Pentostam® 80 mg/kg intramuscularly daily for 14 days (antimony group), FLZ orally at a daily dose of 20 mg/kg for 14 days (FLZ group) or both Pentostam® and FLZ for 14 days (combination group) at the above mentioned doses. The last group of six animals was selected randomly to receive no treatment (control group). The pharmacokinetics of antimony was determined for a single dose (following the first day of treatment) and for multiple doses (following the last dose on Day 14 of treatment). Following drug administration, 0.5 mL of blood was collected from the orbital venous plexus from each group at 0.08, 0.25, 0.5, 1, 2, 3, 4, 6, 8 and 24 h. Animals were anaesthetised with CO_2 during blood sampling. Six animals from each group were bled at each sampling time and each hamster was bled only four times to avoid damage to the eye. Blood samples were maintained at 4 °C until the concentration of Sb^V could be determined. In the single-dose study, blood collection was performed after the first dose from the antimony and combination groups to give two sets of blood samples named Group 1 and Group 2, respectively. In the multiple-dose study, blood collection was performed on Day 14 of daily dosing from the antimony and combination groups to give two sets of blood samples named Group 3 and Group 4, respectively.

2.5. Measurement of infected footpads

Animals were checked daily for survival and overall status. The infected footpads were measured at 2-week intervals from the date of infection using a micrometer.

2.6. Blood analysis of antimony using atomic absorption spectrometry

The antimony concentration in plasma was measured by flameless atomic absorption spectrophotometry, using a

modification of the method used by Veiga et al. [9]. A 2% (v/v) solution of Triton X-100 was used to dilute each blood sample to 1:20. After vortex mixing for 30 s and centrifugation at 100 × *g* for 5 min, 0.5 mL of the clear supernatant solution was run through an AA-680 spectrophotometer under the conditions recommended by the manufacturer (Shimadzu, Kyoto, Japan). The antimony detection limit of the instrument was 8 ng/mL.

2.7. Pharmacokinetic analysis

The pharmacokinetic parameters were determined using model-independent methods [10]. The peak plasma concentration (C_{max}) and the time to reach C_{max} were determined by visual inspection of the plots of concentration versus time. Non-compartmental analysis of the plasma concentrations using the PCNONLIN computer program (Statistical Consultants, Lexington, KY) was performed to determine the area under the concentration–time curve (AUC) for these plots. Elimination rate constants (K_{el}) were determined from linear regression of the last three data points on each of the plots, and plasma half-lives ($t_{1/2}$) were calculated as $0.693/K_{el}$. The area under the first moment curve (AUMC) and mean residence time (MRT; equivalent to AUMC/AUC) were also calculated. The apparent total clearance (CL) was estimated from the equation $CL/F = \text{dose/AUC}$, where F is the bioavailability of antimony following intramuscular administration. The apparent volume of distribution at steady state (V_{ss}) was estimated from the relationship $V_{ss}/F = \text{MRT} \times \text{CL}$.

2.8. Statistical analysis

Unpaired *t*-test was used to investigate differences in each pharmacokinetic parameter in each different dosing study. A P-value < 0.05 was considered statistically significant.

3. Results

The mean Sb^V blood concentration–time profiles obtained after single and multiple dosing of 80 mg/kg alone and with FLZ are depicted in Fig. 1. The mean pharmacokinetic parameters of Sb^V alone and with FLZ are presented in Table 1.

In the single-dose study, the plasma concentration of Sb^V rapidly peaked ($T_{max} = 0.42$ h) at mean peak concentrations of 124.2 ± 25.2 and 144.1 ± 34.3 μg/mL for antimony alone and when administered with FLZ, respectively. A secondary peak was observed ca. 4 h after administration in both groups. Plasma concentrations of Sb^V fell rapidly, with a half-life of 1.84 ± 0.36 and 1.5 ± 0.5 h for the antimony and combination groups, respectively. There was no significant difference in the pharmacokinetic parameters of Sb^V when administered as a single dose alone or in combination with FLZ (Groups 1 and 2, respectively). A two-tailed *t*-test showed no statistically significant differences in C_{max}, K_{el}, CL or V between the above two groups ($P > 0.05$).

In the multiple-dose study, C_{max} for the combination group (Group 4) was significantly ($P < 0.05$) higher (285.14 ± 9.7 μg/mL) than the antimony group (Group 3) (97.1 ± 40.1 μg/mL). Sb^V was eliminated in the combination group more slowly than in the antimony group, with mean

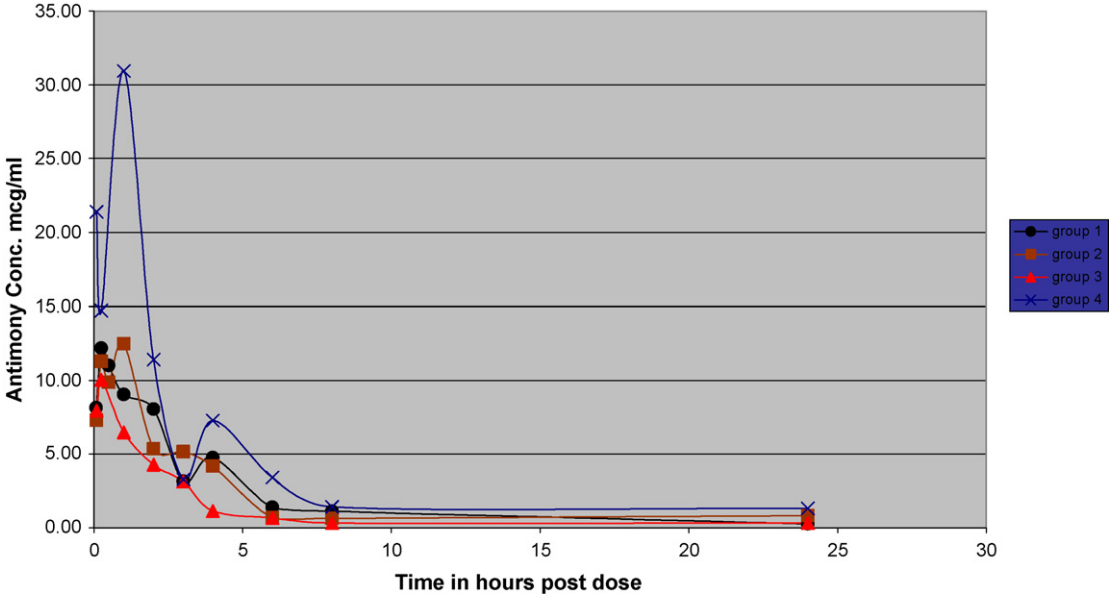

Fig. 1. Mean concentration of antimony (μg/mL) following single and multiple intramuscular administration of Pentostam® 80 mg/kg with and without fluconazole. Group 1, single-dose study, antimony group; Group 2, single-dose study, combination group; Group 3, multiple-dose study, antimony group; Group 4, multiple-dose study, combination group.

Table 1
Mean (± standard deviation) pharmacokinetic parameters in hamsters ($n = 18$) for pentavalent antimony (Sb^V) following intramuscular administration of Pentostam® (80 mg/kg) as single and multiple doses with and without fluconazole (FLZ) 20 mg/kg orally

Parameter	Single dose		Multiple dose	
	Sb^V	Sb^V + FLZ	Sb^V	Sb^V + FLZ
C_{max} (μg/mL)	124.2 ± 25.2	144.1 ± 34.3	97.1 ± 40.1	285.14 ± 9.7*
T_{max} (h)	0.42 ± 0.3	0.71 ± 0.3	0.40 ± 0.3	0.25 ± 0.0
K_{el} (h^{-1})	0.391 ± 0.09	0.46 ± 0.11	0.44 ± 0.09	0.086 ± 0.024*
$t_{1/2}$ (h)	1.84 ± 0.36	1.6 ± 0.50	1.63 ± 0.37	8.67 ± 2.63*
$AUC_{0-\infty}$ (μg h/mL)	449.6 ± 59.2	487.1 ± 132.4	274.9 ± 38.5	1144.6 ± 205.24*
$AUMC_{0-\infty}$ (μg h^2/mL)	2191.7 ± 548.8	2931.1 ± 628.5	1427.0 ± 284.1	11740.01 ± 5219.2
MRT (h)	4.8 ± 0.79	6.20 ± 0.94	5.17 ± 0.53	9.8 ± 3.2*
CL (L/h/kg)	0.18 ± 0.03	0.176 ± 0.05	0.296 ± 0.044	0.072 ± 0.013*
V_{ss}/F (L/kg)	0.863 ± 0.12	1.11 ± 0.43	1.53 ± 0.22	0.68 ± 0.16*

C_{max}, peak plasma concentration; T_{max}, time to C_{max}; K_{el}, elimination rate constant; $t_{1/2}$, plasma half-life; AUC, area under the concentration–time curve; AUMC, area under the first moment curve; MRT, mean residence time; CL, total clearance; V_{ss}, volume of distribution at steady state; F, bioavailability of antimony following intramuscular administration.
* $P < 0.05$.

Table 2
Mean footpad sizes (cm) of *Leishmania*-infected hamsters in different treatment groups throughout the treatment period

Treatment group	Day 1	Day 14	% Reduction from control
Control	0.28 ± 0.02	0.72 ± 0.1	–
Antimony	0.29 ± 0.03	0.55 ± 0.15	23.60
Fluconazole	0.28 ± 0.04	0.6 ± 0.19	16.67
Combination	0.29 ± 0.02	0.67 ± 0.15	6.94

values for $t_{1/2}$ of 8.67 ± 2.63 and 1.63 ± 0.37 h, respectively ($P < 0.05$). In addition, there was a significant reduction in the systemic clearance of antimony in the combination group compared with the antimony group (0.072 ± 0.013 L/h/kg versus 0.296 ± 0.044 L/h/kg) ($P < 0.05$). A secondary peak in plasma concentration was also observed ca. 4 h after dose administration in the multiple-dose study.

The efficacy of the different treatments was assessed by the size of the infected footpads. Two weeks post-infection, the mean footpad sizes were: control group, 0.72 ± 0.1 cm; antimony group, 0.55 ± 0.15 cm; FLZ group, 0.6 ± 0.19 cm; combination group, 0.67 ± 0.15 cm. Footpad sizes of the control group did not differ from any of the treatment groups ($P > 0.05$). Mean footpad sizes were not significantly different across the three different treatment groups ($P > 0.05$). The mean lesion size in each group with the percent reduction in footpad size compared with control is reported in Table 2. A 23% reduction in footpad size was seen in the antimony group, whilst reductions of only 16 and 6% were seen in the FLZ and combination groups, respectively.

4. Discussion

More than 80% of Sb^V is cleared by urinary excretion [11–13]. However, no reports were found to indicate whether active tubular secretion has a role in Sb^V elimination. AL-Jaser et al. [12] reported Sb^V renal clearance in patients infected with CL to be 211.7 ± 19.3 mL/min, which shows possible involvement of active tubular secretion in addition to glomerular filtration. Accordingly, the significant increase in AUC and decrease in CL/F of Sb^V following multiple dosing could be due to FLZ competition with Sb^V renal excretion in hamsters infected with CL. These results are in agreement with the previous report of Belloli et al. [14] who found that aminosidine significantly increased the AUC of Sb^V following subcutaneous administration to dogs. The effect was attributed to a possible interference of aminosidine with the transmembrane transport of Sb^V.

Another possible explanation for the increase in AUC and decrease in CL/F of Sb^V following multiple dosing could be the effect of FLZ on its metabolism. In humans, 10% of Sb^V is converted to trivalent antimony (Sb^{III}) [15]; the percentage in hamsters is unknown. Zaghloul and AL-Jaser [16] found 36.8% (±7.4%) of the dose excreted in urine 72 h after administration, indicating that metabolism may have a larger role in the elimination of Sb^V. Being a metabolism inhibitor, FLZ could have inhibited the metabolism of Sb^V and increased the AUC and C_{max} and decreased the CL/F of Sb^V.

Pharmacokinetic parameters of Sb^V in humans have been described previously [11,12] and are similar to those measured in hamsters in this study. A consistent finding was the appearance of a second peak in the plasma concentration at ca. 4 h after administration. This can be explained by the fact that the drug is injected in the muscle, which may act as a depot for drug release over time. Another interesting observation was the decrease in AUC and C_{max} of Sb^V by 39 and 21%, respectively, after multiple dosing compared with a single dose. We rationalise that because the drug is given intramuscularly, repeated administration can damage the muscle, which might compromise absorption leading to reduced systemic availability.

Efforts to find new effective and safe oral agents for the treatment of leishmaniasis have been ongoing for several decades in order to overcome problems with antimonials. In

this study we used FLZ, an orally active triazole, with lower toxicity and easier administration than antimonials.

We expected that the therapeutic effect of Sb^V and FLZ would be visibly seen in this model, but unfortunately FLZ did not appear to be as effective as Sb^V. Furthermore, the combination of the two drugs did not show better therapeutic response that Sb^V alone even though the concentration of Sb^V was higher.

We observed that the efficacy of treatments was not represented accurately in this experimental model for CL. It was found to be inadequate in assessing response to therapy owing to many factors, including the long treatment period required to detect an appreciable reduction of footpad size. In addition, the infected footpad usually develops secondary infection and necrosis during treatment, which hinders accurate determination of the exact size. In a study of *L. amazonensis* infections treated with KY62, a new polyene antifungal, the difference in lesion size between treated and untreated mice was not observed until 20 days after the treatment period [17]. Moreover, the appropriate dose of FLZ has not been established and the dose used in this study may have been subtherapeutic, therefore, formal studies to address this issue are needed.

Human data on the use of FLZ in CL are deficient and mainly consist of diverse case reports. A placebo-controlled trial of 6 weeks of FLZ for the treatment of CL caused by *L. major* showed complete healing at 3 months follow-up in 63/80 in the FLZ group and 22/65 with placebo [8]. Only 14% compared with 53% in the placebo group were offered Sb^V owing to treatment failure. The study concluded that FLZ is effective and produced faster healing compared with placebo. Limitations of this study were enrolment of only patients with a single lesion, which usually have a high rate of spontaneous healing (34% in the placebo group), or amenable to topical treatments [18]. The efficacy in multilesion disease and the comparative efficacy of Sb^V are not clear.

The results of this study have consistently shown the existence of a potential pharmacokinetic interaction between FLZ and Sb^V, thus warranting caution in their combined use. Extrapolation of the study results to clinical settings must be done with caution. Hamsters and humans may differ markedly in the way that they process Sb^V. In addition, the role of FLZ as an antileishmanial could not be determined because the model used in this study may not represent an ideal model to test antileishmanial activity.

References

[1] World Health Organization. Control of the leishmaniases. Technical report series no. 793. Geneva, Switzerland: WHO; 1990.
[2] Davies CR, Kaye P, Croft SL, Sundar S. Leishmaniasis: new approaches to disease control. BMJ 2003;326:377–82.
[3] Moskowitz PF, Kurban AK. Treatment of cutaneous leishmaniasis: retrospectives and advances for the 21st century. Clin Dermatol 1999;17:305–15.
[4] Hepburn NC. Management of cutaneous leishmaniasis. Curr Opin Infect Dis 2001;14:151–4.
[5] Arevalo I, Ward B, Miller R, et al. Successful treatment of drug-resistant cutaneous leishmaniasis in humans by use of imiquimod, an immunomodulator. Clin Infect Dis 2001;33:1847–51.
[6] Momeni AZ, Jalayer T, Emamjomeh M, et al. Treatment of cutaneous leishmaniasis with itraconazole. Randomized double-blinded study. Arch Dermatol 1996;132:784–6.
[7] Pirson P, Leclef B, Trouet A. Activity of ketoconazole derivatives against *Leishmania mexicana amazonensis* within mouse peritoneal macrophages. Ann Trop Med Parasitol 1990;84:133–9.
[8] Alrajhi AA, Ibrahim EA, De Vol EB, Khairat M, Faris RM, Maguire JH. Fluconazole for the treatment of cutaneous leishmaniasis caused by *Leishmania major*. N Engl J Med 2002;346:891–5.
[9] Veiga JP, Wolff ER, Sampaio RN, Marsden PD. Renal tubular dysfunction in patients with mucocutaneous leishmaniasis treated with pentavalent antimonials. Lancet 1983;ii:569.
[10] Gibaldi M, Perrier D. Pharmacokinetics. 2nd ed. New York: Marcel Dekker; 1982.
[11] Berman JD, Gallalee JF, Gallalee JV. Pharmacokinetics of pentavalent antimony (Pentostam) in hamsters. Am J Trop Med Hyg 1988;39:41–5.
[12] AL-Jaser M, el-Yazigi A, Kojan M, Croft SL. Skin uptake, distribution, and elimination of antimony following administration of sodium stibogluconate to patients with cutaneous leishmaniasis. Antimicrob Agents Chemother 1995;39:516–9.
[13] AL-Jaser M, el-Yazigi A, Croft SL. Pharmacokinetics of antimony in patients treated with sodium stibogluconate for cutaneous leishmaniasis. Pharm Res 1995;12:113–6.
[14] Belloli C, Crescenzo G, Carli S, et al. Disposition of antimony and aminosidine combination after multiple subcutaneous injections in dogs. Vet J 1999;157:315–21.
[15] Chulay JD, Fleckenstein L, Smith DH. Pharmacokinetics of antimony during treatment of visceral leishmaniasis with sodium stibogluconate or meglumine antimoniate. Trans R Soc Trop Med Hyg 1988;82:69–72.
[16] Zaghloul IY, AL-Jaser M. Effect of renal impairment on the pharmacokinetics of antimony in hamsters. Ann Trop Med Parasitol 2004;98:793–800.
[17] Al-Abdely HM, Graybill JR, Bocanegra R, et al. Efficacies of KY62 against *Leishmania amazonensis* and *Leishmania donovani* in experimental murine cutaneous leishmaniasis and visceral leishmaniasis. Antimicrob Agents Chemother 1998;42:2542–8.
[18] Zvulunov A, Klaus S, Vardy D. Fluconazole for the treatment of cutaneous leishmaniasis. N Engl J Med 2002;347:370–1.